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FEEDING SELECTIVITY AND ASSIMILATION
OF PAH AND PCB IN *DIPOREIA* spp.GAIL A. HARKEY,^{††} MICHAEL J. LYDY,[§] JUSSI KUKKONEN^{||} and PETER F. LANDRUM^{*†}[†]Great Lakes Environmental Research Laboratory, 2200 Commonwealth Boulevard, Ann Arbor, Michigan 48105[‡]The Institute of Wildlife and Environmental Toxicology, Clemson University,

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Abstract—A series of experiments were conducted to estimate assimilation efficiencies of two hydrophobic organic contaminants and the influence of particle sizes on the selective sediment ingester, *Diporeia* spp. Florissant soil was divided into particle-size fractions consisting of 0 to 63 μm , 0 to 20 μm , and 20 to 63 μm and dosed with radiolabeled 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP) and/or benzo[a]pyrene (BaP) to which animals were exposed for individual assays. At the end of timed exposure intervals, individual *Diporeia* and any fecal pellets they produced were removed from the sediment and analyzed for contaminant content. Assimilation efficiency was estimated via a selectivity index based on organic carbon. Assimilation efficiency of BaP (5.6–32.7%) was comparable to previous studies. However, HCBP did not correlate with organic carbon over a range of particle sizes. Accumulation of both contaminants was greatest when animals were exposed to the 20- to 63- μm size fraction, suggesting that *Diporeia* selectively fed within this particle-size range. Accumulation of HCBP was consistently greater than BaP in all dual-labeled assays, suggesting a greater bioavailability of the PCB to *Diporeia*. Sediment analysis indicated that BaP and HCBP were associated with different particle-size fractions possessing different amounts of organic carbon, with BaP having the greater tendency to associate with TOC.

Keywords—Assimilation efficiency Bioavailability Sediment *Diporeia* spp. Organic carbon

INTRODUCTION

Neutral hydrophobic compounds such as PAHs and PCBs readily sorb to organic particulate matter and tend to accumulate in sediment. Resistance of these chemicals to biodegradation extends their residence time in the benthic environment. The various routes of contaminant uptake that influence the transfer of sediment-associated contaminants to benthic organisms depend on feeding behavior and characteristics of the sediment and contaminants. Accumulation of sediment-associated contaminants may occur either via the aqueous phase or through ingestion of contaminated particles. Accumulation via the ingestion route depends on feeding rate, assimilation efficiency, gut retention time, and contaminant concentration on the ingested food particles. Some estimates have been made concerning the assimilation of carbon and potassium analogues in aquatic species [1–5] and assimilation of hydrophobic organics by aquatic invertebrates [6–9]. Methods to determine assimilation efficiency have included direct measurement, using TOC as a tracer [8,9]; the dual radiotracer approach that uses the ratio of an assimilated to an unassimilated radioisotope [7,9–11]; and estimation from the ratio of the rate of excretion of absorbed radiotracer to the total rate of excreted radiotracer [4,6]. Assimilation efficiency is defined as accumulation efficiency, or the ratio between the amount of compound absorbed

through the gut and the amount ingested [4]. Each method has produced a relatively wide range of efficiencies among individuals of the same species. Therefore, the processes by which invertebrates assimilate sediment-sorbed contaminants and the factors that influence these processes require additional study to better define the role of ingestion in the bioaccumulation process.

Selective-feeding benthos consume particles with higher organic carbon concentrations and smaller size than non-selective feeders [7,12,13]. In general, these fine materials (<63 μm) contain the bulk of the sorbed organic contaminants and have been identified as major sources of contaminants for Great Lakes benthos [14]. Accumulation of hydrophobic organics in benthos is inversely proportional to the amount of organic carbon present in the sediment [15], thereby creating a trade-off in the amount of contaminant available for uptake and assimilation. The amphipod *Diporeia* spp. (formerly *Pontoporeia hoyi*) [16] is a discriminating feeder, feeding on fine-grained sediment particles [9] and accumulating a substantial fraction of its organic contaminant concentration through ingestion [17,18]. In addition to being a discriminating feeder, *Diporeia* is an intermittent feeder, either feeding on sediment and producing numerous fecal pellets or not feeding [19]. Because fecal pellets in this species are packaged inside a peritrophic membrane and individual *Diporeia* do not actively feed on fecal pellets [9], it is relatively easy to examine and count the number of fecal pellets produced from individual animals. Therefore,

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it should be possible to separate the routes of exposure for *Diporeia*. Those not feeding would presumably accumulate contaminant only from interstitial water and direct contact with contaminated particles. The feeding organisms would also accumulate contaminant from ingestion. Thus, assimilation from ingested material could be determined.

Our objectives were to estimate assimilation efficiencies (AEs) for a selective feeding invertebrate, to compare the AEs for a selected PAH and PCB congener simultaneously, and to examine the influence of particle size on AE. We conducted four separate assays. First, we exposed *Diporeia* to sediment dosed with radiolabeled benzo[a]pyrene (BaP). In the second assay, we replicated the first assay, while exposing animals for longer intervals with the addition of a second radiotracer, 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP). In assays 3 and 4, we separated the sediment into different particle-size fractions <20 μm and >20 μm , to determine the AEs of *Diporeia* for both BaP and HCBP while, again, increasing the length of the exposure intervals.

METHODS

Organisms

Diporeia spp. were collected from surficial sediment at a water depth of 29 m in Lake Michigan in the fall, spring, and summer of 1991 to 1992. Organisms were screened from the sediments, transported to the lab in cool lake water, and kept in 3 to 4 cm lake sediment overlaid with 10 cm lake water at 4°C in the dark. *Diporeia* was allowed to acclimate for at least 3 d before the start of the assays.

Sediment

Sediment used in the assays, characterized as a silt loam soil, was obtained from Florissant, Missouri. This material was selected because of its low carbonate content that permitted simplification of organic carbon analysis. Its use also permitted comparison with previous efforts [9], and it has been used as a reference material in previous sediment bioassays [20]. The sediment was wet sieved through a no. 230 standard testing screen with lake water to obtain a particle-size material of <63 μm for use in the first and second assays. This material was further separated into <20- μm -size particles by passing it through a 20- μm nominal pore opening Nitex® (E.A. Case, Andover, NJ) screen. The material not passing through the screen was saved for use in the fourth assay. The resulting sediments were separately dosed with the radiolabeled compounds [^{14}C]BaP (16.2 mCi/mmol, Sigma Chemical Co., St. Louis, MO), [^{14}C]-2,2',4,4',5,5'-hexachlorobiphenyl (HCBP; 12.2 mCi/mmol, Sigma Chemical Co.), and [^3H]BaP (69.0 Ci/mmol, Amersham Ltd., Amersham, UK) in a minimal amount (<0.5 ml) of acetone carrier. Each compound was checked for radiopurity before use by a combination of TLC and liquid scintillation counting (LSC) [21] and was >98.0% pure. Wet sediment:lake water in a 1:1 ratio was dosed and mixed for 4 h at room temperature, then left to stand in the dark at 4°C for 24 h before beginning the assays. After standing 24 h, the overlying water, along with most of the acetone carrier, was removed before the sediment was added to the test systems.

All preparative and analytical procedures were performed under gold fluorescent lights ($\lambda > 500 \text{ nm}$) to avoid PAH photodegradation.

Experimental procedure

Diporeia was exposed to sediments dosed with [^{14}C]BaP or [^3H]BaP and [^{14}C]HCBP in static systems. The first assay used a single radiolabeled tracer in Florissant soil that contained particle-size fractions 0 to 63 μm . The second assay used dual-labeled radiotracers, [^{14}C]HCBP and [^3H]BaP, also in Florissant soil, that contained 0- to 63- μm -particle-size fractions. The same dual-labeled radiotracers were used in the third and fourth assays. Florissant soil was separated into <20- μm -particle-size fractions and >20- μm fractions for use in assays 3 and 4, respectively.

Diporeia was exposed in 50-ml glass centrifuge tubes that contained dosed sediment equivalent to 2 g dry weight and were filled with filtered Lake Michigan water (dosed sediment equivalent to 1 g dry weight per tube was used in assay 1). Tubes that contained dosed sediment and filtered lake water were left overnight at 4°C to equilibrate before the addition of animals. One animal was placed into each tube. Tubes were individually covered with Fiberglass® window screening secured with a rubber band to prevent escape of *Diporeia*. In addition, three control tubes containing contaminated sediment without animals were employed in each assay to determine any possible changes in the contaminant concentration and/or TOC over the term of the experiment. Twelve tubes that contained uncontaminated sediment with *Diporeia* were also used in each assay to ensure that feeding behavior was not altered by the contaminants or the dosing process. All tubes were kept in aquaria filled with aerated lake water at 4°C in the dark.

Approximately 25 tubes were sampled after each of the timed intervals (7–17 tubes were removed at each timed interval in the first assay). In the first and second assays, overlying water was sampled for contaminant concentration via LSC from three randomly chosen tubes after the timed intervals and was found to be the same as background concentrations. Animals and fecal pellets were removed from the sediment and prepared for LSC. Tubes were sampled after 1, 3, 5, and 7 d in the first assay; 3, 7, 10, and 14 d in the second and third assays; and 10 and 14 d in the fourth assay. Feeding organisms were separated from nonfeeding organisms on the basis of fecal pellet number, arbitrarily set at 30. This number was based on an average fecal pellet production rate reported as 10 fecal pellets per day [22], as well as our own observations in sediment-fed *Diporeia*. Thus, if <30 fecal pellets were found in a tube, the organism was assigned to the nonfeeder group. Fecal pellet contaminant concentrations were determined for all organisms designated as feeders. Three control tubes containing animals and undosed sediment were removed at each timed interval and checked for organism appearance and number of fecal pellets.

Sediment from the tubes was combined at the end of the first and last timed intervals and analyzed for contaminant concentrations. In addition, particle mass, contaminant concentrations, and TOC were determined for the different

particle-size fractions of the sediment after each timed interval in assay 2. Contaminant concentrations and TOC were determined on sediment from control tubes containing dosed sediment and no animals at the end of each assay.

Sampling and analyses

At each timed interval, *Diporeia* was removed from the sediment, rinsed in distilled water, blotted dry, weighed, and placed directly into xylene-based scintillation cocktail (3a70b; Research Products International, Inc., Mt. Prospect, IL). *Diporeia* was not purged of gut contents, because previous work demonstrated no significant increase of total radioactivity from nonpurged vs. purged animals [9]. Samples were sonicated for 30 s using a Tekmar (Cincinnati, OH) high-intensity ultrasonic processor and were analyzed for radioactivity on an LKB (Uppsala, Sweden) 1217 liquid scintillation counter. Samples were corrected for quench using the external standards ratio method after subtracting background. Triplicate-dosed sediment samples were analyzed for contaminant concentration, dry-to-wet ratios, and TOC content before each assay and at the end of each sampling period. The dry-to-wet weight ratios for sediment samples were determined by weighing a wet sediment sample and drying at 90°C to constant weight. Contaminant concentration in the sediment samples was determined by placing approximately 100 mg wet sediment directly into scintillation cocktail and sonicating the sample for 2 min (extraction recovery 82–92% for BaP) [9]. Fecal pellets were individually removed from the sediment via micropipette, transferred, weighed, and dried at 90°C for 2 to 3 h to constant weight. Fecal pellets were weighed again for dry-to-wet determinations, then placed directly into scintillation cocktail and sonicated for 60 s. Samples were left to stand in scintillation cocktail for at least 48 h before determining activity. The TOC content of the sediment was determined by drying sediment samples to constant weight and assaying organic carbon on a Perkin Elmer (Norwalk, CT) 2400 CHN elemental analyzer.

Fractionation of the sediment particles was determined by a modified sedimentation technique [23,24] and confirmed by Coulter technique [25]. Approximately 20 g wet sediment was mixed with 1 L filtered Lake Michigan water in a 1-L graduated cylinder at room temperature. Replicate 25-ml water samples were taken at 20-cm depth at 0, 120, 240, and 600 s. After 1,200 and 4,600 s, water samples were taken at a depth of 10 cm. The sampling times and depths were calculated by Stoke's law using 2.6 as the estimated specific gravity of the particles [23]. The dissolved fraction was separated from the <5- μ m fraction by acidification and centrifugation. From each sample, three 2-ml aliquots were analyzed via LSC. The rest of the sample (19 ml) was dried to constant weight at 90°C for mass and TOC determinations.

Calculation of feeding rates and estimation of assimilation efficiencies

Surrogate feeding rates that used elimination data as a representation of mass were calculated for individual feeding organisms (those that produced >30 fecal pellets) as

$$FR = \frac{\text{dry wt. fecal pellets (mg)}}{\text{exposure interval (h)} \times \text{wet wt. of animal (mg)}}, \quad (1)$$

where FR = feeding rate.

The amount of accumulated contaminant due to ingested sediment was estimated from the difference between feeding and nonfeeding animals at each time interval as

$$Ca_f = Ca_t - Ca_w, \quad (2)$$

where

Ca_f = contaminant concentration in organisms due to feeding (dpm g⁻¹ wet weight)

Ca_t = measured total contaminant concentration in organisms (dpm g⁻¹ wet weight)

Ca_w = estimated concentration accumulated from water (dpm g⁻¹ wet weight).

Ca_w was estimated by two methods. The simplest was to use the average concentrations found in nonfeeding organisms (those that produced <30 fecal pellets) as the estimate of contaminant concentration accumulated from all routes except feeding. The second approach for estimating Ca_w was to plot Ca_t vs. fecal pellet mass/organism mass for each timed interval (Fig. 1). The intercept would represent the accumulation from all nonfeeding sources. This assumes that accumulation from all sources except feeding is the same whether the animals feed or not.

AE was calculated for each timed interval as

$$\%AE = \left(\frac{Ca_f}{FR \cdot SI \cdot t \cdot Cs} \right) \cdot 100 \quad (3)$$

where

AE = assimilation efficiency

Ca_f = mean concentration of contaminant in the animal due to feeding, as determined above (dpm g⁻¹ wet weight)

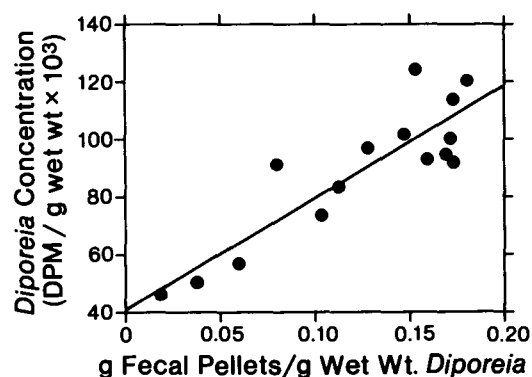


Fig. 1. Plot estimating Ca_w using the y-intercept method. Concentration of BaP in individual animals (Ca_t) was plotted against fecal pellet mass · organism mass⁻¹ for data obtained at day 10 of assay 4. Regression line is $y = 387,457x + 40,876$; $r^2 = 0.77$.

SI = selectivity index, defined as the degree of enrichment of organic carbon in ingested sediment vs. bulk (whole) sediment, and estimated as 6.4 [9]
 t = exposure interval (h)
 C_s = concentration of contaminant in sediment (dpm g^{-1} dry weight).

When AE is calculated from estimates of Ca_f using the difference between Ca_f and the mean of the nonfeeding group, the calculation is referred to as the mean concentration method. When the AE is calculated from estimates of Ca_f determined from the difference between Ca_f and Ca_w estimated from the regression, the method is referred to as the y-intercept method.

The selectivity index used for this work was the mean value from previous studies with *Diporeia* [9] and is based on the feeding selectivity for organic carbon [8].

Calculation of uptake rate coefficients

Accumulation data were fit to a one-compartment model by linear regression [17], using the equation

$$Ca_t = k_s C_s \cdot t, \quad (4)$$

where

k_s = uptake clearance of the contaminant from sediment and interstitial water (g dry sediment g^{-1} wet weight organism h^{-1}).

Two assumptions were made for the initial portion of the exposure periods (0–5 d). First, the concentration of contaminants in the sediment remained constant throughout the course of the assay. Second, the elimination rate and/or biotransformation of the contaminants from *Diporeia* were sufficiently slow and did not result in significant loss over the time course of the exposure. These assumptions are reasonable based on previous measures of *Diporeia* kinetics [17].

Statistics

Differences between feeding organisms (those that produced >30 fecal pellets) and nonfeeding organisms were determined via Student's t tests. Differences were considered significant when $p < 0.05$. Linear regressions were performed with the linear regression packages in SAS® [26] and Microsoft® Excel [27].

RESULTS

No significant differences in accumulation of BaP appeared between feeders and nonfeeders until day 5 of assay 1 (Table 1). Accumulation of BaP in assay 2 was lower in both feeders and nonfeeders than in assay 1, and significant differences in accumulation between feeders and nonfeeders were apparent only after day 10 (Table 1). Accumulation of BaP was very low when *Diporeia* was exposed to the <20- μm -particle-size fraction of sediment (assay 3) and no significant differences in accumulated compound were found between feeders and nonfeeders after any exposure intervals (Table 1). However, significant differences in accumulation of BaP were seen in assay 4, in which *Diporeia* was exposed to the 20- to 63- μm fraction of sediment (Table 1). In assay 4 accumulation of BaP was an average four to 17 times higher on days 10 and 14, respectively, than when organisms were exposed to all particle-size fractions up to 63 μm (assay 2).

Accumulation of HCBP continued to rise through day 14 in all *Diporeia* in assay 2, in which significant differences between feeders and nonfeeders were seen after day 3 (Table 2). However, accumulation of the compound was lower in assay 3, in which organisms (both feeders and nonfeeders) slowly accumulated HCBP to day 10, then declined to day 14. No significant differences in accumulation of HCBP were apparent between feeders and nonfeeders at any exposure intervals in assay 3. Accumulation of HCBP was much greater

Table 1. Accumulation of BaP in *Diporeia*

Assay	Days of exposure	No. of feeders	Accumulation in feeders	No. of nonfeeders	Accumulation in nonfeeders
1	1	6	0.266 (0.08) ^a	4	0.176 (0.07)
	3	4	0.501 (0.13)	3	0.443 (0.086)
	5	9	0.815 (0.14)	7	0.512 ^b (0.084)
	7	12	1.058 (0.29)	5	0.506 ^b (0.09)
2	3	6	0.387 (0.10)	18	0.302 (0.12)
	7	10	0.552 (0.13)	13	0.491 (0.18)
	10	11	0.891 (0.18)	9	0.499 ^b (0.18)
	14	12	0.391 (0.20)	6	0.176 ^b (0.06)
3	3	4	0.093 (0.02)	19	0.083 (0.02)
	7	11	0.208 (0.04)	8	0.181 (0.04)
	10	16	0.262 (0.07)	7	0.225 (0.04)
	14	17	0.243 (0.04)	7	0.233 (0.04)
4	10	16	4.034 (0.58)	3	2.038 ^b (0.21)
	14	13	6.832 (1.73)	4	3.943 ^b (1.47)

Concentrations reported as $ng\ g^{-1}$ wet wt. in *Diporeia*/ $ng\ g^{-1}$ dry wt. sediment.

^a ± 1 SD.

^b Significantly different from feeders at $p < 0.05$.

Table 2. Accumulation of HCBP in *Diporeia*

Assay	Days of exposure	No. of feeders	Accumulation in feeders	No. of nonfeeders	Accumulation in nonfeeders
2	3	5	2.199 (0.86) ^a	18	1.626 (0.96)
	7	6	4.368 (2.04)	14	2.695 ^b (1.06)
	10	11	5.850 (1.85)	9	3.570 ^b (1.84)
	14	9	7.645 (2.94)	6	4.082 ^b (2.24)
3	3	4	1.884 (0.20)	19	1.837 (0.46)
	7	13	2.090 (0.44)	8	2.107 (0.36)
	10	14	3.604 (1.00)	7	3.386 (0.75)
	14	17	3.087 (0.44)	7	3.250 (0.59)
4	10	16	23.600 (3.52)	3	14.578 ^b (1.43)
	14	13	46.297 (13.06)	4	30.085 ^b (10.86)

Concentrations reported as ng g⁻¹ wet wt. in *Diporeia*/ng g⁻¹ dry wt. sediment.

^a ± 1 SD.

^b Significantly different from feeders at $p < 0.05$.

in assay 4, in which accumulation differences between feeders and nonfeeders were readily apparent (Table 2). *Diporeia* accumulated an average of four to six times more HCBP in assay 4 than in assay 2 on days 10 and 14, respectively. Accumulation of HCBP was significantly greater than BaP in all dual-labeled assays (Tables 1 and 2).

Mean feeding rates calculated for each of the four assays (Table 3) did not follow the same trend as contaminant accumulation (Tables 1 and 2). Mean feeding rates ranged from 0.00041 to 0.00264 mg mg⁻¹ h⁻¹ and were highest in assay 1 (Table 3). Control animals taken from uncontaminated sediment produced fecal pellets proportional in number to test animals (e.g., on day 7 of assay 2 the three control animals produced 0, 5, and 133 fecal pellets, indicating the presence of feeding and nonfeeding animals).

The concentration of BaP and HCBP in Florissant soil did not significantly change over the time course of assays 1 to 3, although a significant decrease in BaP concentration occurred between days 10 and 14 of assay 4 ($t = 18.368$, 4 d.f., $p < 0.001$; Table 4). A large variation in sediment concentration of HCBP was observed in assay 4. This may have been due to the high wet-to-dry ratios of the >20-μm fraction (wet-to-dry ratios for individual sediment samples 1.32–2.78) and the difficulty of keeping the >20-μm particles as a homogeneous mixture during sampling. No significant differences in HCBP sediment concentration were seen from days 10 to 14 in assay 4 ($t = 0.921$, 4 d.f., $p > 0.05$). Control sediments without organisms had similar contaminant concentrations as test sediments with organisms present in each of the four assays.

Table 3. Comparisons of uptake rate coefficients and feeding rates for assays 1 to 4 in feeding *Diporeia*

Assay	k_s BaP ^a	k_s HCBP ^a	Days of exposure	Feeding rate ^b
1	0.00557 (0.0007) ^c	NA ^d	1	0.00131 (0.0007)
			3	0.00223 (0.0012)
			5	0.00264 (0.0013)
			7	0.00230 (0.0015)
2	0.00211 (0.0004)	0.01515 (0.0049)	3	0.00180 (0.0005)
			7	0.00094 (0.0005)
			10	0.00124 (0.0005)
			14	0.00112 (0.0010)
3	0.00089 (0.0001)	0.01141 (0.0010)	3	0.00197 (0.0003)
			7	0.00084 (0.0004)
			10	0.00057 (0.0003)
			14	0.00041 (0.0002)
4	NA	NA	10	0.00063 (0.0001)
			14	0.00124 (0.0006)

^a Uptake clearance has units of g dry sed g wet wt. organism⁻¹ h⁻¹.

^b Feeding rate has units of mg dry wt. fecal pellets mg wet wt. organism⁻¹ h⁻¹.

^c ± 1 SD.

^d Not available.

Table 4. Concentration of contaminants in Florissant sediment and fecal pellets in feeding *Diporeia*

Assay	Days of exposure	Sediment		Fecal pellet	
		BaP concn.	HCBP concn.	BaP concn.	HCBP concn.
1	1	349.61 (10.5) ^a	— ^b	1,071.68 (148.8)	—
	3	—	—	495.91 (139.1)	—
	5	—	—	655.18 (411.5)	—
	7	352.38 (7.2)	—	361.98 (91.6)	—
2	3	0.3917 (0.031)	350.08 (79.0)	0.4909 (0.241)	1,917.84 (1616.6)
	7	0.3668 (0.012)	327.66 (53.0)	0.3236 (0.131)	1,998.32 (1402.9)
	10	0.3077 (0.004)	316.06 (59.3)	0.1728 (0.063)	6,776.44 (4133.2)
	14	0.3442 (0.018)	329.35 (90.2)	0.1822 (0.060)	3,191.65 (2553.8)
3	3	0.2233 (0.070)	211.46 (33.0)	0.0680 (0.015)	147.02 (70.9)
	7	0.1892 (0.011)	316.26 (6.0)	0.1054 (0.017)	275.01 (70.4)
	10	0.2022 (0.003)	273.01 (9.5)	0.1129 (0.021)	298.47 (80.0)
	14	0.2357 (0.009)	383.34 (59.8)	0.0851 (0.022)	319.57 (183.3)
4	10	0.0714 (0.031)	56.97 (25.3)	0.3534 (0.077)	952.51 (260.5)
	14	0.0579 (0.031)	43.31 (4.4)	0.2452 (0.131)	686.72 (415.9)

Concentrations are given as ng g⁻¹ dry wt.

^a ± 1 SD.

^b Experiments not performed.

Uptake rate coefficients from sediment (k_s values) for accumulation of BaP in assay 1 were greater by a factor of two to five than for assays 2 and 3, and generally reflected the higher feeding rates in assay 1. The k_s values for BaP were comparable to previous experiments with Florissant soil as the test material [9]. Uptake coefficients for HCBP were greater than those for BaP by a factor of nearly 10. Uptake rate coefficients could not be calculated from data obtained in assay 4, because only two exposure periods were examined (days 10 and 14) at intervals that did not agree with model assumptions. For assays 1 to 3, k_s values were generated from data obtained from days 1 to 7 only, following model assumptions.

In assay 1, the concentration of BaP in fecal pellets (ng g⁻¹ dry weight) was three times that of the sediment concentration after day 1 but dropped drastically over the remainder of exposure intervals to equal that of the sediment concentration on day 7 (Table 4). Fecal pellet concentration of BaP followed the same trend in assay 2, reaching a concentration equivalent to the sediment on day 7. After day 7 fecal pellet concentration continued to drop. However, in assay 2 concentration of HCBP in fecal pellets was over five times greater than the concentration of HCBP in the sediment on day 3. Fecal pellet concentration continued to rise to 21 times that of HCBP sediment concentration by day 10, then dropped at day 14 to 9.7 times the sediment concentration.

In assay 3, in which the <20- μ m-particle-size fraction of sediment was used, the lower concentrations of both contaminants in fecal pellets correlated with the lower accumulation in *Diporeia* (Tables 1 and 4). The concentration of BaP in fecal pellets was consistently lower than it was in the sediment, whereas HCBP concentration in fecal pellets was approximately equal to that of the sediment HCBP concentration. In contrast, where *Diporeia* was exposed to the >20- μ m fraction in assay 4, fecal pellet concentrations of BaP were about

four times that of the sediment concentration, whereas concentrations of HCBP were about 16 times that of the sediment concentration for both days of the study (Table 4).

Particle-size distribution of the Florissant soil used in assay 2 was not significantly different among 3, 7, 10, and 14 d of exposure. The 20- to 31- μ m size class contained a greater portion of sediment mass than the other fractions, whereas the 43- to 63- μ m size class contained the least amount of mass (Fig. 2).

In assay 1 TOC in the sediment dropped from 1.05 ± 0.05% on d 1 to 0.51 ± 0.12% on day 7. Sediment in control tubes without animals contained 1.10 ± 0.01% TOC at the conclusion of assay 1. These results, along with the drop in fecal pellet BaP concentration after day 5, suggest that *Diporeia* may have used up its food supply by the end of the assay. Therefore, twice the amount of sediment used in assay 1 was added to exposure tubes in assays 2 to 4. TOC dropped only slightly from 1.33 ± 0.11% on day 3 to 1.16 ± 0.02% on day 14 of assay 2. Control sediment without animals in assay 2 contained 1.34 ± 0.10% TOC. The TOC dis-

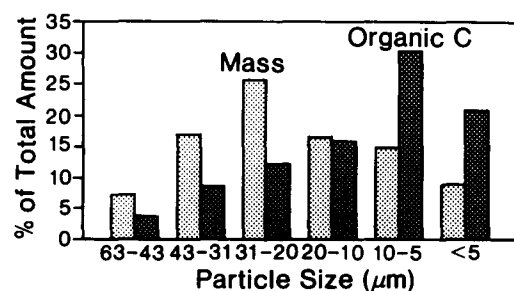


Fig. 2. Particle mass and organic carbon distribution of Florissant soil. Values represent the mean of eight replicates, analyzed on days 3, 7, 10, and 14 of assay 2.

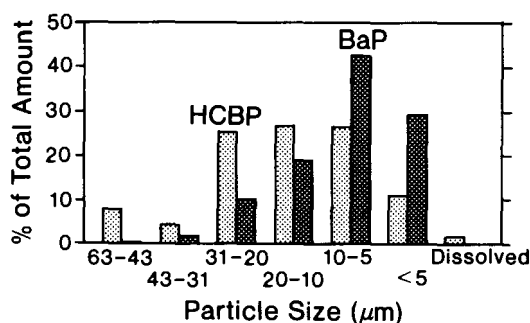


Fig. 3. Distribution of HCBP and BaP in Florissant soil used in assay 2. The dissolved fraction was separated from the <5-μm fraction by acidification and centrifugation.

tribution of the Florissant soil used in assay 2 shows that the 0- to 10-μm-particle-size fractions contained the greatest percentage of TOC, whereas distributions of TOC in the larger size fractions decreased with increasing particle diameters (Fig. 2).

The particle-size distribution for the TOC content of sediment used in assays 3 and 4 was similar to the distribution of carbon shown in Figure 2. Control sediment (without organisms) contained 2.14 ± 0.19 and $0.21 \pm 0.01\%$ TOC for assays 3 and 4, respectively. Although TOC remained relatively constant for the exposure periods examined in assay 4 (TOC = $0.265 \pm 0.06\%$ on day 10 and $0.310 \pm 0.01\%$ on day 14), it increased slightly from $2.13 \pm 0.11\%$ on day 3 to $3.41 \pm 0.11\%$ on day 14 in assay 3.

Contaminant distributions in the sediment for assay 2 suggest that BaP and HCBP distribute differently among the particle-size fractions (Fig. 3). BaP tended to associate more with the <10-μm-size fractions, whereas HCBP tended to associate with the larger particles of 15 to 30 μm.

Because no significant contaminant concentration differ-

ences were apparent among animals designated feeders and nonfeeders for many exposure intervals, it was not possible to calculate a value for Ca_f by the mean concentration method (Tables 5 and 6). However, Ca_f could be determined by the y-intercept method for most of the exposure intervals that allowed AE calculation. In theory, if feeding and nonfeeding animals can be accurately identified, then Ca_w calculated via the mean concentration method should approximate that obtained via the y-intercept method. Ca_w was generally not different between the two methods (Table 6). Where comparisons could be made, the two methods produce similar AE values (Tables 5 and 6). The relatively large standard deviations of contaminant concentrations and assimilation efficiencies shown in Tables 5 and 6 reflect the individual variation among *Diporeia* expressed in all of the assays.

DISCUSSION

Assimilation efficiency determination

Our technique for estimating the assimilation efficiency for BaP from the <63-μm Florissant soil compared favorably with a previous study that examined the assimilation of BaP and calculated AE values (45.9–60.4%) employing TOC as a tracer [9]. The AE for BaP dropped considerably in assay 3, when no differences between feeding and nonfeeding animals could be detected, even though feeding rate was only slightly lowered. Thus, material sorbed to the fine fraction of sediment was not readily bioavailable. The AE values for BaP in assay 4 were generally >100%, suggesting that the source for ingestion was not accurately represented by the bulk sediment concentration. This is especially apparent when the values for assimilation of HCBP are examined for assays 2 and 4 (Table 6).

As the assimilation of any compound cannot be greater than 100%, a reexamination of the terms comprising Equation 3 is in order. In using this equation, AE is calculated from the concentration of compound in the animal (Ca_f)

Table 5. Comparison of BaP assimilation efficiency using mean concentration and y-intercept methods

Assay	Days of exposure	Mean concn. method		Y-intercept method	
		Mean dpm g ⁻¹ nonfeeders, Ca_w	% BaP assimilated	Y intercept, Ca_w	% BaP assimilated
1	1	NA ^a	NA	6,650	29.79 (33.3) ^b
	3	NA	NA	10,963	14.24 (3.1)
	5	13,262 (2,188)	15.26	13,144	20.42 (14.8)
	7	13,113 (2,420)	22.42	13,113	32.67 (14.5)
2	3	NA	NA	52,456	8.57 (3.8)
	7	NA	NA	53,814	11.84 (9.0)
	10	54,074 (19,734)	17.89	88,952	5.55 (4.8)
	14	21,288 (7,728)	15.62	97,051	NA
3	3	NA	NA	37,409	NA
	7	NA	NA	19,198	5.06 (4.3)
	10	NA	NA	19,276	11.51
4	10	51,199 (5,376)	207.60	40,876	238.65 (86.4)
	14	80,319 (30,008)	108.36	78,776	136.66 (88.5)

^aNot available.

^b±1 SD.

Table 6. Comparison of calculated HCBP assimilation efficiency using mean concentration and y -intercept methods

Assay	Days of exposure	Mean concn. method		Y-intercept method	
		Mean dpm g ⁻¹ nonfeeders, Ca_w	% HCBP assimilated	Y intercept, Ca_w	% HCBP assimilated
2	3	NA ^a	NA	51,010	102.69 (65.3)
	7	66,303 (26,202) ^b	164.30	9,673	278.54 (99.2)
	10	84,731 (43,713)	114.10	115,301	79.58 (74.7)
	14	100,953 (55,393)	147.01	115,042	102.88 (86.8)
3	3	NA	NA	22,650	44.15 (20.6)
	7	NA	NA	46,503	63.72 (51.8)
	10	NA	NA	42,733	174.69 (108.6)
	14	NA	NA	85,643	78.80 (96.1)
4	10	62,363 (6,142)	938.27	47,949	1171.23 (372.3)
	14	97,835 (35,325)	608.00	86,036	753.59 (654.4)

^aNot available.^b±1 sd.

after a particular exposure period (t) to a specified contaminant concentration in the sediment (C_s). Because Ca_f is estimated from the difference of Ca_t and our estimate of Ca_w , factors that would result in an underestimate of Ca_w would make Ca_f and therefore AE high. Our study assumed that the uptake for nonfeeders would be representative of all the routes of accumulation except ingestion for feeding organisms. However, the act of feeding may result in more contact with particles or higher respiration that would subsequently increase the relative activity of *Diporeia* and result in a proportionately greater accumulation from other routes than that represented by nonfeeding organisms. Physiological differences in some nonfeeders were exemplified by the molting process. *Diporeia* that had shed their cuticle during the assays almost always produced fewer than 30 fecal pellets. When Ca_t was plotted against amount of fecal material per amount of organism, the value of the y -intercept concentration was generally not different from that measured for nonfeeders. Thus, either measure is believed to be an accurate measure of the accumulation from other routes. If feeding resulted in significant changes in accumulation from other routes, then the y -intercept method would have deviated from the measured. Further, in assay 3 there were no differences between feeders and nonfeeders, again suggesting that feeding does not alter the accumulation from other routes. Therefore, we assume our estimates of Ca_f are reasonable. If an error in calculating assimilation efficiency exists, it must arise in the two remaining terms, feeding rate (FR) and/or selectivity index (SI). The feeding rate was based on fecal pellet production. Because feeding rate values used to calculate BaP assimilation efficiencies are the same as those used for HCBP calculations in the dual-labeled assays (assays 2–4), and BaP assimilation values obtained in assay 2 are comparable to the values estimated in previous studies, the feeding rate term is not the likely source creating the >100% AE values.

The term that is likely responsible for the error resulting in >100% AE is the selectivity index, which adjusts the bulk

sediment concentration to the concentration on the ingested particles. As calculated by Lee et al. [8], SI is the selectivity index for organic carbon (Eqn. 5):

$$SI = \frac{TOC_f / (1 - RC)}{TOC_s}, \quad (5)$$

where

TOC_f = TOC of feces (decimal equivalent)

TOC_s = TOC of sediment (decimal equivalent)

RC = reduction in carbon during gut passage (decimal equivalent).

A basic assumption for the use of the carbon-based SI value is that the contaminant will associate with the organic carbon fraction of feces and sediment. We used an SI of 6.4 to determine the AE of both BaP and HCBP, based on a mean carbon SI from a previous study that estimated the assimilation efficiency of BaP in *Diporeia* [9]. Although this value may be realistic for BaP assimilation in whole sediment, because the BaP distribution is similar to the carbon distribution (Fig. 4), it appears that it must be much greater for HCBP. Part of the failure of a carbon-based SI to describe the relationship between bulk sediment and ingested sediment occurs because HCBP distribution deviates from the TOC distribution in the sediment (Fig. 4). Further, SI values ranging from 46 to 73 would need to be used to obtain a mean AE of 100% for HCBP on day 10 of assay 4. Thus, the concentration on the selectively ingested particles is not determined by TOC alone, but likely includes contributions from differential partitioning and differential ingestion. The differential contaminant concentrations in fecal pellets over the course of the assays (Table 4) may also be due to feeding selectivity changes over the course of the experiments that would affect SI and AE.

An alternative calculation would base SI simply on the ratio of contaminant concentration in fecal pellets to that in the sediment. Assuming no loss of compound during gut pas-

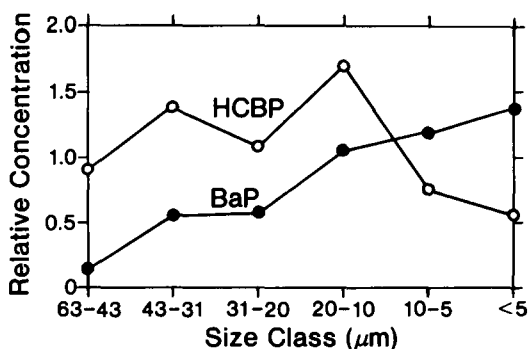


Fig. 4. Relative organic-carbon-normalized concentrations of BaP and HCBP in Florissant soil used in assay 2. Relative concentration = organic-carbon-normalized concentration in size-class fraction \cdot organic-carbon-normalized concentration in bulk sediment⁻¹.

sage, this ratio averages 16 for HCBP in assay 4 (Table 4). This value is still well below the value of 60 needed to obtain a 100% AE alone for 10-d exposures. However, this value may be more realistic if the concentration of HCBP in fecal pellets is artificially low, which may have resulted from loss of compound when drying the fecal pellets at 90°C.

Accumulation and selective feeding in *Diporeia*

The decrease in *Diporeia* contaminant accumulation after day 10 in assay 2 suggested that food resources or the bioavailable fraction of contaminants was depleted. We chose to increase the available fraction in assay 3 by furnishing *Diporeia* the same amount of sediment used in the previous assay (the equivalent of 2 g dry weight) that presumably contained only the "preferred" sediment fraction. According to traditional theory, deposit feeders should select smaller, high-OC particles to maximize their rate of energy gain, based on the assumption that microbes are major food sources that concentrate on sediment particles [28]. Previous studies conducted with *Diporeia* in our lab suggested that fine-grain sediment particles of <63 µm were selectively ingested [15]. Other studies found that *Pontoporeia affinis*, a closely related species, selectively ingested <10-µm-size sediment particles [29,30]. Despite providing *Diporeia* with a presumably preferred food source in assay 3, feeding rates and uptake clearances declined somewhat. Accumulation on day 10 of assay 3 was dramatically lower than in assay 2, in which feeding had a clear influence on accumulation. Because there was no difference in accumulation between feeding organisms and nonfeeding organisms in assay 3, mass feeding rates in assay 3 deviated only slightly from those in assay 2, and contaminant concentration in fecal pellets was similar to contaminant concentration in bulk sediment, then there was no selective feeding and the ingested material was not bioavailable.

In contrast, accumulation of both BaP and HCBP was greatly enhanced when *Diporeia* was exposed to the 20- to 63-µm-size particle fractions used in assay 4, compared to the 0- to 63-µm fractions used in assays 1 and 2, and the 0- to 20-µm fraction in assay 3. In addition to the enhanced accumulation, the mass feeding rate was similar to that in

assays 2 and 3, but the contaminant concentration in fecal pellets was much greater than the contaminant concentration in bulk sediment. These data suggest preferential ingestion among the larger particle range, and, based on the fecal pellet concentration, the preference is for particles that sorb HCBP. Although it is clear that the HCBP preferentially sorbs to the larger fractions relative to BaP (Fig. 4), this is still not sufficient to account for the concentration in the fecal pellets relative to the bulk concentration. Thus, *Diporeia* must be feeding selectively within this particle-size range. Furthermore, the relative concentration between fecal material and bulk sediment for the two contaminants suggests that *Diporeia* selects the particles that contain HCBP and the enhancement cannot be accounted for by the relative partitioning to the various organic carbon fractions. The selectivity for contaminants provides insight into the selectivity for nutritional requirements and suggests that *Diporeia* prefers the material on the larger particles. *Diporeia* has the ability to consume and ingest large particles such as filamentous chains of algae with single-cell lengths of 50 µm [22]. Finally, the decrease in concentration of both BaP and HCBP in fecal pellets from days 10 to 14 in assay 4 also suggests that available food sources were depleted after day 10 in our experimental systems.

The bioavailability of hydrophobic compounds is a function of the amount of carbon in the system [6,31-33]. Although our data suggest that BaP was more closely associated with TOC in the sediment than was HCBP, the decrease in TOC from days 1 to 7 in assay 1 did not correspond with a decrease in sediment BaP concentration (Table 4). That BaP sediment concentration remained relatively unchanged throughout the assay further indicates that TOC is clearly not the sole factor regulating bioavailability of these hydrophobic contaminants.

In our study, uptake rate coefficients declined with increasing sediment TOC (Table 3), consistent with previous studies [15]. The fact that k_e values for BaP and feeding rates were greater in assay 1 than in assay 2, in which sediment TOC was similar, may be due in part to the higher concentrations of BaP in the sediment used in assay 1. Sediment BaP concentrations were about 1,000 times greater in assay 1 than in assay 2. Increases in uptake clearances have previously been seen when *Diporeia* was subjected to sediment dosed with a mixture of PAHs ranging from 41 to 120 nmol g⁻¹ [34] and when exposed to Florissant soil dosed at 1.44 and 42.9 nmol g⁻¹ [9]. The average molar concentration of BaP in the sediment used in assay 1 was only 1.39 nmol g⁻¹, compared with 0.23 to 1.55 pmol g⁻¹ in assays 2 to 4.

Accumulation of both contaminants in *Diporeia* was greatest in assay 4 and may be due to low sediment TOC and differential partitioning of contaminants to the various particle-size fractions. The large accumulation of HCBP compared to BaP in this assay may be the result of both the greater tendency of BaP to be associated with organic carbon and the differential association of contaminants to sediment particles. We have shown that realistic values for the assimilation of BaP in whole sediment could be obtained, assuming that the contaminant associates with organic carbon, whereas values for HCBP could not be determined using this

method. Consequently, additional factors that regulate the exposure of HCBP to *Diporeia* must be involved.

The differential association of the two contaminants to particles shown in Figure 3 indicates that HCBP and BaP are not equally distributed among particle-size classes. If this distribution was similar among particles in the 20- to 63- μ m-size fraction used in assay 4 and *Diporeia* ingested particles mainly from the smaller sizes within this class (i.e., the 20- to 31- μ m-size class), exposure to HCBP would have been greater than to BaP. Further assays that expose *Diporeia* to more extensively separated particle sizes may produce additional insights.

CONCLUSIONS

The data generated in this series of experiments demonstrated that organic contaminants that possess similar solubilities in lipid (i.e., octanol/water partition coefficients) associate differently with organic carbon, which resulted in differential bioavailability to *Diporeia*. Although a method that bases the assimilation of contaminants on organic carbon as a tracer may be useful for some classes of hydrophobic contaminants, it is not valid for contaminants that partition differentially among organic carbon components in the system. Accumulation of the HCBP was consistently greater than the PAH in all dual-labeled sediment assays, suggesting that the chlorinated congener was more bioavailable to *Diporeia* than the unsubstituted aromatic hydrocarbon. The greatest accumulation of both contaminants from the 20- to 63- μ m-particle-size fraction suggests that *Diporeia* prefers sediment of this size. The greater accumulation of HCBP in this size range further supports the idea that *Diporeia* exhibits an extremely selective feeding behavior. These findings should be evaluated further, using a variety of discriminative-feeding species in a range of sediment types, before they are extrapolated to more natural systems.

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